I. EXPERIMENTS ON VACCINATION AGAINST RAT LEPROSY.*†

II. ON THE EXTRACTION OF RAT LEPRA BACILLI FROM WATERY EMULSIONS BY MEANS OF CHLOROFORM.

III. RAT LEPRA BACILLI IN THE RAT LOUSE.

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I.

EXPERIMENTS ON VACCINATION AGAINST RAT LEPROSY.

TECHNIC of preparing the vaccine:

Vaccine I.—Pieces of the subcutaneous and glandular tissues from advanced cases of natural rat leprosy were ground up with powdered glass, extracted with 0.85 per cent sodium chloride solution, and the supernatant fluid pippeted off into a tall cylinder. This was then heated in flowing steam for 30 minutes; when the coagulated albuminins had precipitated, the supernatant, opalescent fluid, rich in lepra bacilli, was removed and autoclaved at 10 lbs. pressure for 30 minutes. When cool it was preserved with 0.5 per cent carbolic acid. An attempt was made to standardize the emulsion by counting the number of lepra bacilli in $\frac{1}{100}$ c.c. of a 1:100 dilution of the uncarbolized emulsion. This showed that vaccine I contained more or less than 20,000,000 bacilli per c.c.

Vaccine II was prepared in the same way, excepting that it was simply heated once for 15 minutes at 20 lbs. pressure. It contained approximately the same number of bacteria per c.c.

1. EXPERIMENTS TO DETERMINE WHETHER VACCINATION WITH DEAD LEPRA BACILLI WOULD INFLUENCE THE COURSE OF INOCULATION LEPROSY IN THE RAT.—White rats weighing 100 gm. were inoculated subcutaneously (abdomen) with an emulsion of rat lepra

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† Castellani attempted the vaccination of human lepers with emulsions prepared from their own tissues but I do not know where the results have been recorded. A similar independent attempt was made by P. G. Woolley in Siam. He, however, was unable to continue the treatment for a sufficient period. (*Proc. Soc. Exper. Biol. and Med.*, 1907, 4, p. 121.)

bacilli on February 20, 1909. This was the third passage through the white rat of a strain obtained from *Mus norvegicus* on February 13, 1908. Ten of these were vaccinated as follows and ten kept as controls. The vaccine was injected subcutaneously to one side or other of median line—the site of infection.

a) Nos. 7 and 8.—Treatment commenced two days after infection, with 5,000,000 dead lepra bacilli. Other injections were as follows:

TABLE 1.

(VACCINE I.)

Number of Injection	Number of Dead Lepra Bacilli Injected	Number of Days Since Last Injection
1	5,000,000	..
2	5,000,000	10
3	5,000,000	14
4	10,000,000	14
5	10,000,000	14
6	20,000,000	18
7	20,000,000	10

b) Nos. 9 and 10.—Treatment commenced 12 davs after infection and received injections numbered 2–7 (Table 1).

c) Nos. 11 and 12.—Treatment commenced 26 days after infection and received injections numbered 3–7 (Table 1).

d) Nos. 13 and 14.—Treatment commenced 38 days after infection and received injections numbered 4–7 (Table 1).

e) Vaccine I had been kept for eight months before it could be used, so Nos. 15 and 16 received injections corresponding to numbers 5, 6, and 7 (Table 1) of vaccine II which was about one month old.

RESULTS.—Rat 9 died four days after the last injection and was found to be infected, and likewise with No. 11 which died nine days after the last injection. The remaining eight vaccinated rats were chloroformed 78 days after the last injection along with an equal number of infected controls. There was practically no difference in the extent to which the disease had progressed in the treated and untreated rats, the area of infection being about 4 cm. in diameter and extending to the inguinal glands.

2. EXPERIMENTS TO DETERMINE WHETHER VACCINATION WOULD PRODUCE ANY IMMUNITY TO SUBSEQUENT INOCULATION.—A large adult *Mus norvegicus* (M.n. 1) (250± gm.) and a white rat (25) (100± gm.) were vaccinated with 15 and 10 million bacilli in two separate injections, 14 days apart, of 10 and 5 million and 5 and 5 million bacilli respectively.

Seven days after the last injection they, along with two controls

of about the same weight (M.n. 3), and white rat 27, were inoculated with 1 c.c. of a broth emulsion of living rat lepra bacilli. They were chloroformed 127 days after the injection of the living lepra bacilli. M.n. 1 showed a slight amount of infiltration at the site of inoculation covering an area of 15 by 5 mm. The inguinal glands were not enlarged. Microscopically lepra bacilli were numerous at the site of inoculation but none were found in smears from the inguinal glands.

White rat 25 showed no signs of infection excepting three scattered whitish nodules, 1–3 mm. in diameter, in the fascia of the abdominal muscles at the site of the inoculation. The 3 mm. nodule contained caseous pus which microscopically showed numerous lepra bacilli. No bacilli could be found in the inguinal glands.

Control M.n. (3) showed an area of leprous infiltration about two inches long by one inch wide at the site of inoculation and its inguinal glands were considerably enlarged. Lepra bacilli were very numerous at the site of inoculation and in the inguinal glands.

Control white rat 27 showed an area of leprous infiltration about three inches by 1.5 inches at the site of inoculation and its inguinal glands were considerably enlarged. Smears showed numerous bacilli in the glands and at the site of inoculation.

Other rats in this series are still under observation.

SUMMARY.—Injection of an emulsion of rat lepra bacilli, killed in the autoclave, in doses varying from 5 to 20 million bacilli at intervals of 10 to 14 days, failed to arrest the progress of inoculation leprosy in the white rat even when treatment was commenced 48 hours after infection.

One *Mus norvegicus* and one white rat received two injections of the vaccine, on the 21st and 7th day respectively, before inoculation with living lepra bacilli. Here the progress of the disease was markedly delayed as compared with its progress in two controls.

II.

ON THE EXTRACTION OF RAT LEPRA BACILLI FROM WATERY EMULSIONS BY MEANS OF CHLOROFORM.

The subcutaneous tissue and glands from a leper rat were ground up with powdered glass and extracted with 0.85 per cent sodium

chloride solution. This clouded emulsion was shaken up with commercial chloroform which took on a clouded appearance. When drops of the chloroform were evaporated and the residue stained it was seen that millions of lepra bacilli had been extracted free from all cellular elements and other bacteria. The possible value of this fact in aiding the diagnosis of human leprosy has not yet been determined. Possibly it might help one in detecting lepra bacilli in the nasal secretions of an early case.

III.

RAT LEPRA BACILLI IN THE RAT LOUSE.

A leper rat (*M. norvegicus*, adult male) in a very advanced stage of the disease was seen to be literally covered with louse eggs. Only a few lice could be found. Six of them (*Haematopinus spinulosus*) were ground up on a slide and stained for lepra bacilli. Several hundred acid proof bacilli resembling the bacillus of rat leprosy were found scattered about in what appeared to be the granular contents of the intestinal tract.

These examinations were made in April, 1909, and are the only ones made on rat lice taken from leper rats since the publication of a previous note.[1] A number of lice from normal rats were examined for acid proof bacilli with negative results.

[1] *Jour. Infect. Dis.*, 1908, 5, p. 509.